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Biological monitoring of cadmium exposure: reliability of spot urine samples

Andrea Trevisan, Giampaolo Nicoletto, Stefano Maso, Guiseppe Grandesso, Alexander Odynets, and Livia Secondin

Laboratorio di Tossicologia Industriale, Istituto di Medicina del Lavoro, Università di Padova, I-35127 Padova, Italy

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Summary. Concentration-dilution of spot urine samples is a shortcoming of the biological monitoring of industrial xenobiotics. To ascertain whether the adjustment of urinary cadmium measured in spot samples is appropriate, urine samples were taken three times, once a week for 3 successive weeks, from 25 welders employed in the manufacture of jewellery (total 75 samples). Cadmium, creatinine, specific gravity, total urinary solutes, urinary volume and urinary flow rate were measured in 12-h collections and in spot samples taken immediately afterwards. Creatinine and total urinary solutes showed high inverse correlation with urinary flow rate (r = –0.858 and r = –0.768 respectively). Urinary cadmium displayed a similar trend but the correlation was not significant (r = –0.145). Creatinine adjustment of urinary cadmium values in spot samples increased the correlation with the same index in timed samples adjusted for urinary volume (r = –0.808) or urinary flow rate (r = 0.821) compared with non-adjustment (r = 0.732 and r = 0.738, respectively). Creatinine adjustment of spot sample values is also suitable for a wide range of urinary concentrations; discarding excessively diluted or concentrated urines, correlation of urine samples improved for non-adjusted or specific gravity-adjusted values, whereas no changes were observed for creatinine-adjusted values.

Key words: Urinary cadmium – Creatinine – Specific gravity – Adjustment

Introduction

Cadmium exposure is usually monitored by means of indices of exposure, such as cadmium excretion in urine (CdU) and cadmium concentration in blood (CdB), and by indices of effect, such as total and low-molecular-weight proteins and kidney enzymes in urine.

Urinary determinations are suitable because sampling is relatively easy and not invasive, but the choice of urine samples is problematic. Urine specimens collected over a long period (12 or 24 h) are unsuitable for biological monitoring of industrial chemicals because: (1) subjects tend to be unwilling to comply, and (2) quickly excreted metabolites will be diluted. In such cases, the collection of spot samples, generally taken at the end of the work shift, is more appropriate. On the other hand, spot samples show high variation due to concentration-dilution of urine. Adjustment of urinary values for a standard specific gravity (SG) of 1024 or creatinine (Cn) concentration is common, but some authors do not agree with the necessity of this adjustment.

The variation of urinary concentration in spot specimens makes non-adjusted values unacceptable (Pryde 1982; Trevisan 1990); on the other hand, Alessio et al. (1985), Berlin et al. (1985) and Dell'Orto et al. (1987) maintain that no practical benefit is obtained when urinary values of cadmium and aminolevulinic acid are adjusted.

The aim of the present study was to ascertain whether urinary cadmium values are more accurate after adjustment for concentration-dilution of urine in spot samples and timed collections.

Materials and methods

Twenty-five subjects (22 females and 3 males) aged 15–30 years (average 22 years) with an exposure duration of 4.6 years (range 1–11 years), employed as welders in jewellery factories, living in the same geographical area and with similar socioeconomic characteristics (alcohol and nicotine consumption, living conditions) were chosen to study variability of urinary cadmium related to concentration-dilution of urine in spot and timed specimens.

The subjects were informed about the aim of the study, and urine was collected for 12 h from Tuesday 8. p. m. to Wednesday 8 a. m. for three successive weeks. A spot sample was collected each Wednesday morning (first urination after the end of the timed collection).

In timed and spot urines (75 samples each), CdU was measured according to Ross and Gonzales (1974) (precision ± 5%, accuracy 100% after two extraction, detection limit 0.1–0.2 μg) and Cn was determined using a commercial kit (Boehringer Mannheim, Germany). SG was measured with an AT 315 Uricon difractometer (Japan), and total urinary solutes (TUS) were calculated from SG and Long's constant (2.6 times the last two digits of the SG value). Urinary flow (UF) rate was also measured.

Correspondence to: A. Trevisan

Table 1. Cadmium concentration (CdU), creatinine (Cn), specific gravity (SG), total urinary solutes (TUS) and urinary flow rate (UF rate; medians and ranges) in the spot samples (s) and timed collections (t) of urine, adjusted or not adjusted (na) for the different parameters used

		First week	Second week	Third week
CdU (µg/l)				
na	t	5.4 (0.6–43)	7.2 (0.8–33)	5.7 (1 –60)
	s	7.2 (0.9–22)	3.1 (0.5–50)	5.7 (0.6–63)
Cn	t	4.4 (0.4–25.5)	4.3 (0.3–25.4)	3.8 (0.5–28.4)
	s	4.9 (0.5–35.3)	7.6 (0.5–54.9)	6.6 (0.3–38.4)
SG	t	7.9 (0.8–57.6)	9.0 (0.9–40.8)	6.6 (0.9–53.3)
	s	11.5 (0.9–42.9)	10.9 (0.9–55.6)	9.1 (0.9–96.0)
Volume	t	2,5 (0.2–17.1)	2.0 (0.3–17.7)	1.8 (0.3–17.0)
$UFCn_r$	t	2.7 (0.2–34.5)	2.2 (0.1–27.1)	2.6 (0.2–28.6)
UF	t	3.2 (0.3–21.7)	4.2 (0.2–22.2)	3.5 (0.3–21.2)
Cn (g/l)				
na	t	1.61 (0.47–3.16)	1.81 (0.52–3.59)	1.51 (0.41–4.33)
	s	1.65 (0.22–4.14)	1.47 (0.26–3.48)	1.12 (0.26–3.84)
Volume	t	0.53 (0.33–0.86)	0.55 (0.30–0.84)	0.60 (0.38–0.93)
UF	t	0.74 (0.40–1.16)	0.75 (0.38–1.34)	0.80 (0.50–1.06)
SG	t	1019 (1005–1031)	1021 (1005–1031)	1024 (1006–1032)
	s	1022 (1005–1031)	1021 (1004–1027)	1019 (1005–1030)
TUS (g/l)				
na	t	49.4 (13.0–80.6)	54.6 (13.0–80.6)	62.4 (15.6–83.2)
	s	57.2 (13.0–80.6)	54.6 (10.4–70.2)	49.4 (13.0–78.0)
Volume	t	20.6 (10.9–34.9)	18.1 (7.5–38.8)	19.5 (10.0–35.9)
UF	t	25.1 (13.5–44.3)	26.6 (9.4–54.6)	27.8 (12.4–46.2)
UF (ml/min)		0.46 (0.26–1.55)	0.48 (0.20–1.17)	0.51 (0.19–2.26)

A Perkin-Elmer 305 atomic absorption spectrophotometer equipped with a graphite furnace HGA 76 and background corrector was used for cadmium determination. A UV-Vis spectrophotometer (Perkin-Elmer lambda 5) was employed for Cn analysis.

Values in spot samples were adjusted, or not, for Cn or for the SG standard of 1024 according to Elkins et al. (1974). Values in timed samples were adjusted for volume, UF rate or, according to Greenberg and Levine (1989), for UF-adjusted creatinine ratio ($UFCn_r$).

Owing to non-normal distribution, the values were presented as medians and ranges; linear regression analysis was used for statistical evaluation of the results on a semilog scale.

Results

Table 1 shows the variation of CdU, Cn, SG, TUS and UF rate in timed and spot samples in the three collections, adjusted or not for concentration-dilution of urine. In general, wide variations are not observed among the three samples. Cn-adjusted specimens show lower values than non-adjusted or SG-adjusted specimens, e.g. timed samples adjusted for urinary volume, $UFCn_r$ or UF rate. In addition, values in spot samples are generally higher than in timed samples.

Table 2. Correlation of Cn and TUS with UF rate (n = 75)

	b (slope)	*r*
Cn	–1.36	–0.858
TUS	–1.46	–0.768

Table 3. Correlation of CdU in spot samples, not adjusted or adjusted for Cn or SG, with CdU in timed collections adjusted for volume, $UFCn_r$ or UF rate

	b (slope)	*r*
Volume		
na	5.64	0.732
Cn	6.36	0.808
SG	5.98	0.756
$UFCn_r$		
na	6.99	0.522
Cn	9.02	0.667
SG	8.20	0.602
UF		
na	7.46	0.738
Cn	8.31	0.821
SG	7.84	0.769

Cn and TUS (Table 2) show high inverse correlation with UF rate (r = –0.858 and r = –0.768, respectively), whereas non-adjusted CdU (r = –0.145) shows the same trend but no significant correlation (data not shown).

The slope of the log-log correlation between Cn excretion rate and UF rate was 0.697, similar to the 0.67 calculated by Araki et al. (1986) and Greenberg and Levine (1989). This number was used as the exponent for the UF rate to calculate $UFCn_r$ according to Greenberg and Levine (1989).

Table 4. Correlation of CdU in spot samples without Cn <0.5 or >3.0 g/l and SG <1010 or >1035 ($n = 64$), not adjusted or adjusted for Cn and SG, with CdU in timed samples adjusted for urinary volume, $UFCn_r$ or UF rate

	b (slope)	*r*
Volume		
na	6.11	0.769
Cn	6.25	0.803
SG	6.20	0.771
$UFCn_r$		
na	7.78	0.617
Cn	8.32	0.673
SG	8.03	0.639
UF		
na	7.99	0.786
Cn	8.14	0.817
SG	8.05	0.794

Correlations between non-adjusted, Cn-adjusted and SG-adjusted CdU values in spot samples and CdU values in timed specimens related to urinary volume, UF rate and $UFCn_r$ are summarized in Table 3. Adjustment of CdU for Cn in spot specimens improves the correlations and shows less scatter of the values. The lower correlations are generally found with $UFCn_r$-adjusted values. Discarding too diluted or too concentrated specimens (Cn < 0.5 or > 3.0 g/l, SG < 1010 or > 1035; Trevisan 1990), correlation improved only for non-adjusted or SG-adjusted values (Table 4).

Discussion

Urinary volume (Araki 1978) and UF rate (Araki et al. 1990) affect excretion of the majority of substances in urine; for this reason, Araki (1980) introduced a mathematical equation that is applicable to virtually all urinary substances under conditions of wide variation in urinary volume (Araki et al. 1986). Greenberg and Levine (1989) suggested another equation to adjust Cn excretion for UF rate, because Cn excretion is not stable and is dependent on UF rate. The inconvenience of this model is the necessity of timed sample collection, which is difficult in biological monitoring for the reasons explained above.

In the present study, excretion of substances in three different collections was similar between timed and spot urines, adjusted or not for Cn, SG, volume, UF rate and $UFCn_r$. Urinary excretion of substances was generally similar from day to day. Adjustment for Cn further reduced differences among values.

The decreases in Cn and TUS with increasing UF rate confirm previous data (Greenberg and Levine 1989; Araki et al. 1990). CdU also shows the same trend, and although the changes are not significant, this supports Cn adjustment. Furthermore, adjustment for urinary Cn improves correlations between values measured in spot samples and values in timed samples adjusted for volume and UF rate. In contrast, values in timed samples adjusted for $UFCn_r$ show worse correlation with non-adjusted and adjusted values in spot urines than do values in timed samples adjusted for volume or UF rate. Therefore $UFCn_r$ adjustment appears not to be suitable for biological monitoring.

In general, the results confirm previous observations (Trevisan 1990) indicating that spot samples are suitable for biological monitoring of cadmium exposure when adjusted for Cn.

If CdU values measured in excessively diluted or concentrated samples are discarded, the correlation between values determined in spot samples and those in timed collections adjusted for volume or UF rate is improved only for non-adjusted or SG-adjusted values. This evidence shows that adjustment for Cn is justified in a wide range of concentrations, whereas non-adjustment and adjustment for SG are suitable only for the suggested range.

Finally, because adjustment for SG does not improve the accuracy of values measured in spot urine samples and does not avoid the influence of concentration-dilution of urine, this adjustment is of no benefit in determining CdU.

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